510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number: k040350

B. Purpose for Submission:
Premaket Notification [510(k)] of intention to manufacture and market the Amphetamine Class Assay Test, to include Amphetamine and Methamphetamine for use on the evidence® Analyzer.

C. Analyte: Amphetamine, Methamphetamine

D. Type of Test: Qualitative competitive chemiluminescent immunoassay for the detection of amphetamines in urine.

E. Applicant: Randox Laboratories Ltd.

F. Proprietary and Established Names:
Randox Amphetamine Class Assay for use on the evidence® Analyzer and evidence® Drugs of Abuse Calibrators

G. Regulatory Information:

1. Regulation section: 21CFR §862.3100 - Amphetamine test system
   21 CFR §862.3610 – Methamphetamine test system
   21 CFR §862.3200 – Drug Mixture Calibrators

2. Classification: Class II

3. Product Code: DKZ, LAF, DKB

4. Panel: Toxicology (91)

H. Intended Use:

1. Intended use(s):

The evidence® Amphetamine assays is an in vitro diagnostic test for the qualitative determination of Amphetamine Class of compounds (including amphetamines and methamphetamine), central nervous system stimulating drugs, in human urine. This is a competitive immunoassay. A primary cut-off of 1000 ng/ml has been established in accordance with SAMHSA recommendations.

The evidence® Drugs of Abuse Calibrators are liquid Calibrators containing benzoylecgonine, amphetamine and methamphetamine. There ae 9 levels of calibrator. They have been developed for use in calibration of the evidence® system.

This assay is for use only on the automated evidence® Analyzer.

Note: This test provides only a preliminary analytical result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas Chromatography/ Mass Spectrophotometry (GC/MS) is the preferred confirmatory method.

The Amphetamine Class Assay and Calibrators must only be used by suitably qualified laboratory personnel under appropriate laboratory conditions.

2. Indication(s) for use:

The evidence® Amphetamine Assay and Drugs of Abuse Calibrators compose a set of in vitro diagnostic tests for the qualitative determination of amphetamine and methamphetamine, central nervous system stimulating drugs, in human urine. This is a competitive immunoassay. A primary cut-off of 1000 ng/ml has been established in line with SAMHSA recommendations. Qualitative results obtained can be utilized in the diagnosis and treatment of amphetamine use or overdose.

The evidence® Drugs of Abuse Calibrators are used in distinguishing positive from negative samples. A normalized value is calculated as a percentage of the signal intensity emitted from the cut-off test region relative to the signal intensity emitted from the sample test region. Cut-offs of 1000 ng/mL d-amphetamine and methamphetamine are recommended for the evidence® Amphetamine Class Assay.

3. Special condition for use statement(s):

These tests provide only a preliminary analytical result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas Chromatography/ Mass Spectrophotometry (GC/MS) is the preferred confirmatory method. The Amphetamine Class Assay must only be used by suitably qualified laboratory personnel under appropriate laboratory condition

4. Special instrument Requirements:

The evidence® Amphetamine Assay is designed for use on the Randox evidence® Automated Immunoassay Analyzer.

I. Device Description:

The Randox Laboratories Limited Amphetamine Class Assay includes Drugs of Abuse Assay Diluent, Drugs of Abuse Conjugate, and Drugs of Abuse Biochips. Materials required by not provided are evidence® Wash Solution, evidence® Displacement Fluid, evidence® Signal Reagent, and evidence® Drugs of Abuse Calibrators.

J. Substantial Equivalence Information:

1. Predicate device name(s): Microgenics Corp. Cedia Dau Amphetamine Assay

2. Predicate K number(s): k941372

3. Comparison with predicate:
These in vitro diagnostic tests are automated competitive enzyme immunological assays. The two differ in that in the proposed device the antibodies have been attached to a microchip in such a way that multiple drugs of abuse test can be conducted on the same sample simultaneously. These devices are qualitative assays, which provide a preliminary analytical test result. In both cases a more specific alternative chemical method must be used to obtain confirmed analytical results, for example gas chromatography/ mass spectrometry (GC/MS). The new device is for use specifically with the Randox evidence® Analyzer.

K. Standard/Guidance Document Referenced (if applicable):
Interference screening to identify exogenous and endogenous compounds was done in accordance with the NCCLS EP7-A. Total imprecision of the Evidence Amphetamine Class test kit on the Randox Laboratories Ltd evidence® Analyzer was done in accordance with NCCLS EP5-T2.

L. Test Principle:
The evidence® Analyzer is a fully automated Biochip Array System. It performs simultaneous detection of multiple analytes from a single patient sample. The core technology is the Randox Biochip, a solid-state device containing an array of discrete test regions containing immobilized antibodies specific to different DoA compound classes. A competitive chemiluminescent immunoassay is employed for the DoA assays. The drug in the specimen and drug labelled with horseradish peroxidase (HRP) directly compete for antibody binding sites on the chip. Increased levels of drug in a specimen will lead to reduced binding of drug labelled with HRP and thus reduce the chemiluminescence of the test region.

The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to the signal from a stored calibration curve. A normalized value is calculated as a percentage of the signal intensity emitted from the cut-off point on the calibration curve relative to the signal intensity emitted from the sample test region. Samples producing a response value greater than, or equal to, the response value of the calibrator cut-off is considered positive (normalized result ≥ 100). Samples producing a response value less than the response value of the calibrator cut-off is considered negative (normalized result < 100).

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. Precision/Reproducibility:

Total imprecision of the evidence® Amphetamine Class Assay on the Randox Laboratories Ltd evidence ® analyzer was done in accordance with NCCLS EP5-T2 analysis of variance. Calibrators covering the assay cut-off ranges were tested two times in the same run, twice a day for 20 days to generate a total of 80 replicates. At least 10

patient samples were included in each run, which were separated by a minimum of 2 hours.

All data points were incorporated in the results. No results were rejected as outliers unless procedural or analytical errors occurred. Total imprecision was conducted on evidence® analyzers at Randox Laboratories Ltd, Northern Ireland and VANTHCS, Dallas, TX.

Total imprecision results are presented as normalized values in the tables below:

Amphetamine assay

Site 1

Concentration (ng/mL d-Amphetamine)	558.2	762.1	959.2	1121.6	1465.1
Mean (Normalized)	60	76	92	107	137
SD	6.0	7.2	9.3	10.1	12.1
%CV	10.0	9.5	10.0	9.5	8.8

Site 2

Concentration (ng/mL d-Amphetamine)	558.2	762.1	959.2	1121.6	1465.1
Mean (Normalized)	61	78	93	111	135
SD	6.3	8.2	10.2	13.8	13.8
%CV	10.3	10.6	10.9	12.4	10.2

Methamphetamine assay

Site 1

Concentration (ng/mL Methamphetamine)	431.2	630.5	765.5	1021.9	1588.1
Mean (Normalized)	50	62	81	102	158
SD	5.2	5.6	8.5	14.9	18.8
%CV	10.5	9.0	10.5	14.4	11.9

Site 2

Concentration (ng/mL Methamphetamine)	431.2	630.5	765.5	1021.9	1588.1
Mean (Normalized)	52	67	81	100	159
SD	5.7	9.0	9.3	9.3	16.6
%CV	11.0	13.4	11.4	9.3	10.5

b. Linearity/assay reportable range:

Not applicable. This test is for qualitative determinations.

c. Traceability (controls, calibrators, or method):

The concentration of d-amphetamine and (+)-methamphetamine in each of nine calibrator levels was GC/MS assigned by an independent College of American Pathologist (CAP) approved laboratory.

Aliquots of these calibrators were stored at -80 °C for reference purposes while the remainder was held at the recommended storage temperature of (+2-8 °C). After 1 year at (+2-8 °C) these calibrators were compared to those stored at -80 °C for the same period of time. Calibrator sets stored at -80 °C and (+2-8 °C) were directly compared. A stability

of 1 year at (+2-8 °C) was assigned where the % difference in either % B/Bo (where B is the rlu for an individual calibrator level and Bo is the rlu for the level 1 calibrator) or normalized values between the -80 °C and (+2-8 °C) was <10%. Real time stability results support expiration dating in the labeling.

Open vial stability of the evidence® Amphetamines test kit calibrator was assessed by opening and closing a series of calibrator vials daily to replicate normal usage. Values assessed at day 14 were compared to day 0 materials. The assay reagents were considered stable to 14 days if there was ± 10% deviation from day 0 results.

d. Detection limit:

The sensitivity of the evidence® Amphetamines Class test kit was established by analyzing 20 repeat determinations of a GC/MS verified negative urine sample. The mean normalized value was calculated and 2 standard deviations were added. The resultant normalized values of 22 and 26 for amphetamine and methamphetamine, respectively, represent the lowest concentration which can be distinguished from the zero calibrator with a confidence level of 95%. The equivalent concentration for this relative light unit can be calculated from the Amphetamines Class calibration curves.

d. Analytical specificity:

The specificity and cross-reactivity of the evidence® Amphetamine Class Assay were assessed using a dose-response series based on a 1000 ng/mL cut-off for d-amphetamine and methamphetamine in accordance with SAMHSA recommendations. Each compound was diluted in GC/MS verified negative urine to the ranges specified. Compounds listed were tested in duplicate to a maximum of 0.5 mg/ml. Concentrations of the cross-reactants, which produce a response equal to that of the target compound at the cut-off, were calculated. Results are presented below:

Specificity/Cross-reactivity for Randox Amphetamine Class assay

	Amphetamine Assay		**Methamphetamine Assay**	
Compound	**Crossreactant Concentration (ng/mL)**	**% Cross - Reactivity**	**Crossreactant Concentration (ng/mL)**	**% Cross - Reactivity**
d-Amphetamine	1000	100	1000	1.0
Methamphetamine	>500,000	<6.0	1000	100
dl-Amphetamine	1818	55	250,000	0.4
MDA	183.8	544	200,000	0.4
MDMA	250,000	<6.0	2778	34
MDEA	>500,000	<6.0	176393	5.8
BDB	529	189	>9000	<11
MBDB	833	120	1124	89
Fenfluramine	>500,000	<0.2	5263	19
Phentermine	3125	32	>500,000	<0.2

More than 45 structurally unrelated drugs were evaluated for potential interference by spiking into negative urine; they, and the concentrations at which they were tested, are listed in the package insert.

A variety of commonly used over-the-counter and prescription drugs and endogenous compounds were tested for interference by spiking into negative urine and measuring the percent difference between the control sample (no potential interferents) and the test sample (containing potential interferents). Results are presented below:

Amphetamine Class assays: Common Substance Interference screening

	Amphetamine Assay		**Methamphetamine Assay**	
Compound	**Concentration tested (mg/dL)**	**% difference**	**Concentration tested (mg/dL)**	**% difference**
Acetaminophen	1 mg/mL	-1.65	1 mg/mL	0.27
Acetone	1000	-1.95	1000	-3.05
Acetylsalicyclic acid	1 mg/mL	-2.92	1 mg/mL	-1.77
Ascorbic Acid	1500	-9.65	1500	-1.96
Caffeine	1 mg/mL	-4.19	1 mg/mL	-2.58
Creatinine	500	-1.00	500	-0.12
Ethanol	1000	-1.00	1000	-1.15
Galactose	10	-1.31	10	-3.19
Gamma globulin	500	-1.25	500	-2.30
Glucose	3000	-0.12	3000	2.77
Hemoglobin	300	-1.38	300	0.92
Human serum albumin	500	-0.38	500	-0.26
Ibuprofen	1 mg/mL	-3.50	1 mg/mL	-4.73
Oxalic acid	100	-1.88	100	1.98
Ranitidine	0.9 mg/mL	3.42	5 ug/mL	7.40
Riboflavin	7.5	3.63	7.5	4.58
Sodium chloride	6000	-2.23	6000	-3.00
Urea	3500	-2.35	3500	-1.15

e. Assay cut-off:

The performance of the evidence® Amphetamine Class Assay was assessed using cut-offs of 1000 ng/mL d-amphetamine and methamphetamine in accordance with SAMHSA recommendations. Ten GC/MS verified commercial controls at 25% below the cut-off, at the cut-off and 25% above the cut-off were analyzed for each of the two assays. The mean normalized value, the number of determinations, the standard deviation, the percentage coefficient of variation. Samples with drug concentrations at 25% below the cutoff were all negative and samples with drug concentrations above the cutoff were all positive.

Cut-off Concentration for Amphetamine assay

	75% cutoff	100% cutoff	125% cutoff
Mean	87.7	114.8	136.9
Range	75 - 95	103 - 126	128 - 145
SD	6.4	8.1	4.6
%CV	7.3	7.1	3.4
N	10	10	10

Cut-off Concentration for Methamphetamine assay

	75% cutoff	100% cutoff	125% cutoff
Mean	85.9	112.1	130.1
Range	73 - 95	101 - 129	110 - 144
SD	6.9	8.5	11.0
%CV	8.0	7.5	8.4
n	10	10	10

2. Comparison studies:

a. Method comparison with predicate device:

Method comparisons were conducted at an external laboratory. Results were compared using Randox Laboratories Ltd evidence®, Hewlett-Packard gas chromatography/mass spectrophotometer (GC/MS) and Microgenics Cedia DAU test kits on an Olympus AU600.

1336 clinical neat urine samples were selected randomly by the trial site to ensure that drug concentrations present in the samples covered the entire range of possible test results for all drugs of abuse assays. Results were compared primarily using Cedia immunoassay system followed by GC/MS confirmation when requested by the test site laboratory (borderline/ positive sample or discrepancy between methods). Since Cedia DAU Amphetamine test kit cannot differentiate between amphetamine and methamphetamine assays, 63 and 78 samples were not included for the amphetamine and methamphetamine assays respectively when Cedia was compared with evidence® or GC/MS. In all cases, evidence® data agreed with GC/MS. A further 8 sample were also not included in the Cedia comparisons with the evidence® or GC/MS as the combined amphetamine and methamphetamine levels exceeded the cut-off. These samples would therefore produce a positive screen with the Cedia DAU Amphetamine test kit. Again in all 8 cases, evidence® data agreed with GC/MS.

Particular emphasis was applied near stated cut-off concentrations. For GC/MS comparison 39 and 23 samples, distributed between 25% above the cut-off and 25% below the cut-off concentration of the assay, were tested for the amphetamine and methamphetamine assays respectively. A 1000 ng/mL cut-off was selected thus giving approximately a 750 – 1250 ng/mL range for the ± 25% cut-off. Within the 1336 sample analysis, 1051 and 1054 samples were determined negative for amphetamine and methamphetamine by the evidence® and Microgenics Cedia DAU Amphetamine test kit. These negative samples included 100 samples that were selected using Microgenics

Cedia DAU as being negative for all drugs of abuse assays and of these 10 samples (10%) were confirmed negative by GC/MS.

Summary comparison of Evidence® with Cedia

		CEDIA	
		+	-
d-Amphetamine Evidence® 1000 ng/mL cut-off	+	174	5
	-	35	1051
		CEDIA	
Methamphetamine Evidence® 1000 ng/mL cut-off		+	-
	+	156	2
	-	38	1054

306 samples were tested by GC/MS for amphetamines and methamphetamines and compared to evidence® results. Eight of the 17 discrepant amphetamine results were within ±25% of the cutoff. Two of the nine discrepant methamphetamine results were within +25% of the cutoff.

Summary comparison of Evidence® with GC/MS

		GC/MS	
		+	-
d-Amphetamine Evidence® 1000 ng/mL cut-off	+	170	16
	-	1	119

		GC/MS	
		+	-
Methamphetamine Evidence® 1000 ng/mL cut-off	+	152	6
	-	3	145

b. Matrix comparison:

Not applicable. This device is indicated only for urine specimens.

3. Clinical studies:

a. Clinical sensitivity:

Not applicable. Clinical studies are not typically submitted for this device type.

b. Clinical specificity:

Not applicable. Clinical studies are not typically submitted for this device type.

c. Other clinical supportive data (when a and b are not applicable):

3. Clinical cut-off:

Analytical characterization of performance around the cut-off was demonstrated in the precision studies.

5. Expected values/Reference range:

Not applicable.

N. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.